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Toxicologic effects of ribavirin in cats

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Ribavirin, a broad-spectrum antiviral agent active *in vitro* against a number of RNA and DNA viruses, has been associated with moderate toxicity in laboratory animals and humans. Clinically, ribavirin has been used effectively in persons primarily to treat life-threatening viral diseases such as acute haemorrhagic fever or viral pneumonia of infants. In order to evaluate the feasibility of using this antiviral agent in cats, the effects of oral (p.o.), intramuscular (i.m.) and intravenous (i.v.) doses of ribavirin in 27 9-month-old specific-pathogen-free cats were evaluated by haematology, clinical chemistries, bone marrow biopsies and histopathology. Ribavirin was administered once daily for 10 consecutive days at a dose of either 11, 22, or 44 mg/kg after which all cats were euthanatized and necropsied. Most cats receiving 22 or 44 mg of ribavirin/kg became anorectic and suffered some degree of weight loss (0.2 to 0.6 kg), and about one-third of the cats developed diarrhoea and/or mucous membrane pallor. Icterus or haemorrhage was not observed. The most profound and consistent haematologic change, particularly among the moderate and high dosage groups regardless of route of administration, was a significant and severe thrombocytopenia (range, 33–78% reduction in mean platelet counts vs. baseline). Other changes, particularly reductions in total WBC and neutrophils and reductions in RBC and PCV, tended to occur at lower ribavirin dosages, but generally they were not statistically significant. Cats given 44 mg of ribavirin/kg i.v. showed significant decreases in leukocyte variables, including total WBC ($P = 0.016$), neutrophils ($P = 0.026$) and lymphocytes ($P = 0.047$). Mild-to-moderate increases in serum alanine aminotransferase and alkaline phosphatase activities occurred at doses of 22 and 44 mg/kg. Evaluation of bone marrow biopsies before and after treatment revealed that cats given 11 mg of ribavirin/kg had mild megakaryocytic (MK) hypoplasia, whereas cats receiving 22 or 44 mg/kg had progressively severe degrees of MK hypoplasia and dysplasia, asynchronous MK maturation, and increased myeloid:erythroid ratio. Pathologic changes in ribavirin-treated cats generally were mild and included primarily enteritis (seven cats) and hepatocellular vacuolation and/or centrilobular necrosis (seven cats). Results of this study in cats indicated that daily administration of ribavirin at a dose range of 11 to 44 mg/kg induced a dose-related toxic effect on bone marrow, primarily on megakaryocytes and erythroid precursors, and at the higher dosages it suppressed numbers of circulating leukocytes.

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INTRODUCTION

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide; Virazole), a nucleoside analogue related to guanosine, has broad-spectrum virostatic activities *in vitro* against many DNA and RNA viruses, including herpesviruses, adenovirus, poxvirus, influenza

type A and B, respiratory syncytial virus (RSV), parainfluenza virus, measles, arenavirus, bunyavirus, and the human immunodeficiency virus, HIV (Sidwell *et al.*, 1972; McCormick *et al.*, 1984). Because of potential toxicity, the clinical application of ribavirin in humans has been limited mostly to aerosol therapy of infants with serious RSV infection, or i.v. or p.o. administration of adults with influenza A or acute viral haemorrhagic fever (e.g., Lassa, Junin, Machupa) (Reines & Gross, 1988; Bryson, 1988). Results of clinical trials of oral ribavirin in HIV-infected patients have been equivocal (Roberts *et al.*, 1990; Bodsworth & Cooper, 1990).

There have been few published antiviral studies of ribavirin against viruses of veterinary importance. *In vitro* studies have demonstrated that ribavirin has significant antiviral activities against canine parainfluenza virus, feline calicivirus (FCV) (Povey, 1978a) and feline infectious peritonitis virus (Weiss and Oostrom-Ram, 1989). Ribavirin had significant cytotoxicity ($ID_{50} \approx 17$ μg/ml) in rapidly proliferating normal feline cells, and the resultant low therapeutic index (6.8) suggested potential toxicity when used at antiviral dosages in cats (Weiss & Oostrom-Ram, 1989). Experimentally, ribavirin administered orally to cats at a divided total daily dose of 75 mg/kg starting 1 or 4 days after exposure to FCV failed to have any beneficial effect on the clinical course of disease or reduce virus excretion (Povey, 1978b). The ribavirin treatment was associated with severe toxic effects including thrombocytopaenia, decreased red and white blood cell counts, increased alanine aminotransferase activity, haemorrhage, and loss of body weight. In laboratory animal species, various other toxic effects of ribavirin occurred in both acute and chronic toxicity studies, including teratogenicity and embryotoxicity in rabbits and rats, gastrointestinal necrosis and bleeding in beagles, skin scaling and ulceration of the tail base in rats, alopecia in rabbits, and decreased food consumption, body weight and testicular and seminal vesicle weights in rats (Canonico, 1983; Hillyard, 1980; Gillett *et al.*, 1990).

Our purpose was to evaluate the effects of ribavirin administration on the health of specific-pathogen-free cats as measured by haematology, clinical chemistries, bone marrow, and histopathology. Because previous studies on the clinical use of ribavirin in cats had demonstrated toxicity and lack of efficacy at dosages of ribavirin used successfully in other animal species (Povey, 1978b), we were interested in evaluating the toxicity of ribavirin at a range of dosages lower than that used previously in cats. Potentially, this data will be useful in helping define dosages of ribavirin tolerated clinically in cats infected with various viruses, including feline leukemia virus (FeLV) or feline immunodeficiency virus (FIV). These viruses, particularly FIV, may serve as valuable animal models for the study of human AIDS and for evaluation of antiretroviral agents (Pedersen, 1989). Additionally, we wished to understand the pathogenesis of ribavirin toxicity in cats so that strategies may be designed to combat its adverse effects.

MATERIALS AND METHODS

Experimental animals

Twenty-seven healthy 9-month-old specific-pathogen-free cats of either sex weighing 3.47 ± 0.28 kg (mean ± SD) were obtained from a commercial breeder (Harlan Sprague Dawley, Inc., Austerlitz, The Netherlands). These cats were FeLV antigen-negative (by ELISA) and were seronegative for feline coronavirus and FIV antibodies. All cats were housed individually in isolation rooms in stainless steel cages with food and water provided *ad libitum* and they were used and maintained according to US federal guidelines. Experimental protocols were approved by the Auburn University Institutional Animal Care and Use Committee.

Ribavirin

The ribavirin (Virazole; lot no. 03100785-[n-15], Viratek Inc, Costa Mesa, CA, USA) was provided by Dr D. Hines (Solvay Animal Health, Inc, Mendota Heights, MN, USA) and Dr B. Rossiter (ICN Pharmaceuticals, Inc, Costa Mesa, CA, USA). The drug initially was stored lyophilized (50 g) at room temperature until diluted prior to use. Ribavirin solutions were made by mixing a predetermined quan-

tity of lyophilized powder with phosphate buffered saline solution (PBSS) on a magnetic stirrer for 10 min then filtering the solution through a 0.2 μm cellulose acetate membrane filter (Corning Inc, Corning, NY, USA). The solutions were stored at 4°C for a maximum of 2 weeks until use. *In vitro* screening of representative aliquots of the ribavirin stored for several weeks or more at 4°C for antiviral activity against feline coronavirus, using a previously described procedure (Weiss & Oostrom-Ram, 1989), confirmed drug stability under these storage conditions.

Experimental design

The cats were assigned randomly to one of three experimental groups (nine cats/group) based on ribavirin dosage as follows: Group I (11 mg/kg), Group II (22 mg/kg), or Group III (44 mg/kg). Each group was subdivided further into three subgroups (3 cats/subgroup) depending upon the particular route of administration, i.e., p.o., i.m., or i.v. Before starting treatment of cats with ribavirin on post-treatment day (PTD) 0, each cat was sedated with ketamine hydrochloride (Vetalar; Parke, Davis & Co, Detroit, MI, USA; 11 mg/kg, i.m.), weighed, and a sample of bone marrow collected aseptically from the ilium. Approximately 2.7 ml of blood was then collected by jugular venipuncture into vacuum-evacuated blood tubes containing 0.06 ml of 75% EDTA (K_3) or glass tubes without anticoagulant (Vacutainer tubes, Becton-Dickinson, Rutherford, NJ, USA) for determination of complete blood count and platelet numbers or serum chemistries, respectively. Ribavirin then was administered to each cat by the appropriate route once daily for 10 consecutive days. For p.o. administration, the drug was administered slowly into the oral cavity, using a plastic 3-ml syringe without needle. For i.m. administration, the drug was injected deeply into the semimembranosus muscle. For i.v. administration, each cat was sedated with ketamine and catheterized daily by inserting a 23 gauge butterfly catheter (Sherwood Medical, St. Louis, MO, USA) into the cephalic vein; then, the ribavirin was injected slowly into the catheter and the catheter flushed with sterile PBSS.

Cats were weighed twice weekly and they were monitored daily for clinical signs of toxicosis. Amounts of drug administered were adjusted as necessary to compensate for changes in body weight. A clinical score, which was correlated inversely with the health of an animal, was calculated daily for each cat on the basis of a 10-point numerical scale reflecting severity of clinical signs. Daily clinical scores were determined according to the following grading of signs: anorexia = 7; fever (39.5 C–39.8 C) = 6; fever (≥ 39.9 C) = 7; hypothermia (≤ 37.5 C) = 8; depression = 7; pale mucous membranes and/or slow capillary refill time = 8; dehydration = 8; vomiting = 8; diarrhoea/melena = 8; weight loss (0.25–0.4 kg) = 7; weight loss (> 0.4 kg) = 8; laboured breathing = 8; CNS signs (seizure, tremor, incoordination, paresis/paralysis etc.) = 9; death = 10. On PTD 10, each cat was sedated and blood sampled for testing. An additional bone marrow sample was collected. Cats were subsequently euthanatized by an i.v. injection of a solution containing pentobarbital sodium and phenytoin sodium (Beuthanasia®-D Special, Schering-Plough Animal Health, Kenilworth, NJ, USA) and were necropsied on PTD 10 or 11.

Haematologic evaluations

Complete blood counts, including total white blood cell count (WBC) and differential, red blood cell count (RBC), packed cell volume (PCV), and haemoglobin (Hb) were performed by semi-automated methods (Coulter S550, Coulter Electronics, Inc., Hialeah, Florida). Platelet counts were performed using an automated instrument (Baker series 810, Baker Instrument Corp, Allentown, PA, USA) within 30 min of blood collection. Serum chemical and electrolyte parameters were determined with commercially available kits using automated instrumentation (Cobas Mira, Roche Diagnostic Systems, Nutley, NJ, USA and Beckman E4A, Beckman Instruments, Somerset, NJ, respectively). Parameters evaluated included alanine aminotransferase (ALT), alkaline phosphatase (SAP), creatine kinase (CK), creatinine, glucose, blood urea nitrogen (BUN), total anionized calcium and phosphorus, total pro-

tein (TP), albumin, globulin, sodium, potassium, chloride, total carbon dioxide, and anion gap.

Bone marrow evaluation

Bone marrow samples were aspirated from the ilium at times corresponding with blood collection. Samples were collected into syringes containing 54 mM EDTA-0.15 NaCL and they were placed immediately into plastic petri dishes. Bone marrow particles were isolated with a microhematocrit tube and microbulb, expressed onto glass slides, and gently spread with another slide. Slides were stained with a modified Wright's stain. Bone marrow smears were evaluated for cell morphology, cellularity, and maturation sequence. A myeloid:erythroid (M:E) ratio (minimum of 500 cells) was calculated for each sample.

Necropsy examination

Two cats given 22 mg of ribavirin/kg i.v. died shortly after ketamine was administered on PTD 6 or 9 (necropsy examination did not reveal immediate cause of death); the other 25 cats were euthanatized and necropsied on PTD 10 or 11. All gross lesions and representative sections of brain, liver, lung, heart, kidneys, adrenals, stomach, small and large intestines, urinary bladder, thymus, spleen, mandibular, retropharyngeal and mesenteric lymph nodes, gonads and bone marrow were fixed in 10% neutral buffered formalin. Tissues were embedded in paraffin, sectioned at 6μm, and stained with haematoxylin and eosin for histopathologic evaluation.

Statistical analysis

Comparisons of pre- and post-treatment haematologic and biochemical values were performed using the Student's *t*-test on paired samples and a computerized statistical analysis program (Abstat; Anderson-Bell, Canon City, CO, USA). Because the clinical scores collected from each treatment group were not distributed normally, the Mann-Whitney *U*-test for nonparametric data was used to evaluate the clinical scores. The paired Student's *t*-test (2-tailed) was used to evaluate mean differences in percentage change of values pre- and post-treatment between treatment groups at each dosage level. The % change was calculated as the difference in values pre- and post-treatment divided by the pre-treatment value, multiplied by 100. Values of $P \leq 0.05$ were considered significant.

RESULTS

Clinical responses

Other than mild weight loss (range, 0.2–0.5 kg), adverse signs were not observed in cats receiving 11 mg of ribavirin/kg. Most cats given 22 mg of ribavirin/kg lost some weight (range, 0.2–0.6 kg), and several of these cats given the drug p.o. or i.v. also developed mild pallor of mucous membranes, starting at PTD 5 or 6. One cat given ribavirin at this dosage developed transient diarrhoea starting at PTD 5; two others died while under ketamine anaesthesia, one cat at PTD 6 and the other at PTD 9. Cats receiving 44 mg of ribavirin/kg consistently demonstrated the most severe signs, including anorexia, weight loss (range, 0.3–0.6 kg), depression, intermittent diarrhoea, and mucous membrane pallor. Icterus or haemorrhage was not observed. Anorexia occurred in eight of nine cats given 44 mg of ribavirin/kg and ranged in duration between 1 and 7 days, usually beginning at PTD 3 or 4. The diarrhoea, which generally was mucoid, occurred in three of the nine cats (2 i.m., 1 i.v.). The clinical scores of cats given 44 mg of ribavirin/kg i.m. or i.v. were significantly higher ($P \leq 0.05$) than scores of cats given 11 or 22 mg of ribavirin/kg by all routes (Fig. 1). At a dosage of 44 mg/kg, the clinical scores of cats administered ribavirin p.o. were significantly less ($P = 0.040$) than scores of cats given ribavirin i.v. and were similar ($P = 0.095$) to scores of cats given ribavirin i.m. (Fig. 1).

Haematologic and blood chemistry changes

Cats given 11 mg of ribavirin/kg had a relative decrease in mean Hb, PCV and RBC

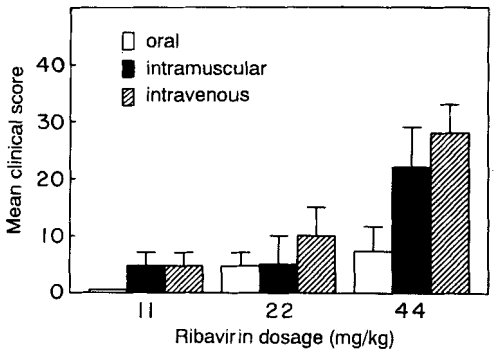

FIG. 1. Mean clinical scores (± SD) of cats treated for 10 days with ribavirin at various dosages by p.o., i.m., or i.v. route. The height of each bar is proportional to severity of disease signs. Each bar represents the mean score of three cats.

values compared to mean baseline values (significant at $P < 0.05$ for the i.m. group; Table I). Between treatment groups, relative decreases in Hb, PCV, and RBC were greatest in the p.o. group and least in the i.v. group. Cats given ribavirin p.o. or i.m. had a mild decrease (approximately 20%) in WBC numbers. Neutrophil counts after treatment were significantly ($P = 0.049$) reduced in the p.o. group; there were no differences, however, in the magnitude of decrease post-treatment in numbers of neutrophils between groups. Mean platelet numbers were reduced by 33% in i.v.-treated cats. A significant decrease in blood calcium ($P = 0.03$) along with a concomitant decrease in phosphorus occurred in all groups (Table II). Total protein was significantly ($P = 0.03$) decreased in cats given ribavirin p.o. ($P < 0.05$ vs. relative decrease in TP for i.m. and i.v. groups). Blood glucose concentrations were reduced in the i.m. and i.v. groups (significant for i.v. group, $P = 0.02$). Blood sodium concentrations were significantly ($P < 0.05$) reduced in all groups. The anion gap was reduced in all three groups. Creatinine, BUN or serum enzymes were not significantly increased in cats after ribavirin treatment.

The most marked and consistent haematologic disturbance in cats receiving 22 mg of ribavirin/kg was a decrease in platelet count (range, 42–58% mean decrease vs. baseline; significant for p.o. and i.v. routes) accompanied by an increase in mean platelet volume (Table III). Platelet counts in cats given ribavirin i.v. were significantly decreased compared to counts in cats given ribavirin p.o. ($P = 0.01$) or i.m. ($P = 0.05$). Cats given ribavirin at 44 mg/kg i.v. had a decrease in WBC numbers and showed a significant decrease ($P = 0.05$) in numbers of lymphocytes. Serum ALT was mildly but significantly increased ($P = 0.05$) in cats given ribavirin orally and the SAP was significantly increased ($P = 0.029$) in cats treated with ribavirin i.v. (Table IV). The SAP levels were significantly increased in the i.v. group vs. the oral ($P = 0.01$) or i.m. groups ($P = 0.009$). Blood calcium levels were reduced ($P = 0.001$) in the oral group. Blood sodium concentration was mildly increased in all groups ($P < 0.05$ for the oral group), and the anion gap was significantly ($P < 0.05$) increased in the p.o. and i.m. groups. Creatinine and BUN values were unchanged. Two cats (A4 and A6) that were given ribavirin i.v. and had died during the treatment period were sampled within 1–2 h of death. However, samples for determinations of ALT, glucose, phosphorus, potassium, total CO_2, and anion gap were unsuitable, and these data could not be included in statistical evaluations.

Cats receiving 44 mg of ribavirin/kg had significant reductions in platelet counts (range, 38–78% mean decrease vs. baseline) regardless of the route of administration (Table V). However, cats given ribavirin p.o. had significantly greater reductions in platelet numbers than those given ribavirin i.m. or i.v. ($P = 0.038$). Increases in mean platelet volume accompanied the thrombocytopenia. Generally, haematologic changes other than thrombocytopenia, including significant decreases in WBC ($P = 0.016$), neutrophils ($P = 0.026$) and lymphocytes ($P = 0.047$), were most severe in the i.v. group. Mean WBC and neutrophil counts decreased between 30–60% of baseline values in cats given ribavirin p.o. or i.m., but these changes were not statistically significant. Mean ALT and CK levels were increased after treatment in all three groups; significant changes in ALT, however, were observed only in the i.m. group ($P = 0.035$) and the i.v. group ($P = 0.007$), whereas significant increases in CK occurred only in the i.v. group ($P = 0.046$; Table VI). Blood calcium and phosphorus levels were decreased significantly ($P = 0.031$ and 0.036, respectively) in the p.o. group; calcium levels,

TABLE I. Haematologic alterations in cats associated with the administration of ribavirin (11 mg/kg) by p.o., i.m., or i.v. routes

	p.o.		i.m.		i.v.	
Variables	Pre	Post	Pre	Post	Pre	Post
Haemoglobin (g/dl)	13.9 ± 1.0*	11.1 ± 1.2	12.7 ± 0.5	10.7 ± 0.7†	13.3 ± 0.4	12.5 ± 0.4
PCV (%)	42 ± 3.5	34 ± 3.6	38.3 ± 0.9	33 ± 1.5†	41 ± 0.6	38.7 ± 1.3
Erythrocytes ($\times 10^6/\mu l$)	9.3 ± 0.6	7.7 ± 0.9	9.2 ± 0.2	7.9 ± 0.3†	8.8 ± 0.4	8.3 ± 0.3
Leukocytes ($\times 10^3/\mu l$)	6.8 ± 1.1	5.5 ± 1.2	8.8 ± 2.0	7.2 ± 1.6	7.7 ± 0.5	8.0 ± 2.1
Neutrophils ($\times 10^3/\mu l$)	4.0 ± 0.5	2.1 ± 0.7†	5.0 ± 1.6	3.2 ± 1.2	5.1 ± 0.5	4.4 ± 1.5
Lymphocytes ($\times 10^3/\mu l$)	2.5 ± 0.7	3.3 ± 1.2	3.2 ± 0.6	3.4 ± 0.5	2.4 ± 0.6	3.2 ± 0.4
Platelets ($\times 10^3/\mu l$)	119.3 ± 40	125 ± 45.4	111 ± 27.1	112.7 ± 34.2	123 ± 56	84.3 ± 40.2
Mean platelet volume (fl)	12.2 ± 0.4	13.4 ± 0.1‡	11.7 ± 0.2	11.8 ± 0.1	12.5 ± 0.1	13.9 ± 0.9

*Values represent mean ± SE (n = 3 cats for each route) of all observations. Cats were sampled 24 h before administration of ribavirin (pre) and again after 10 days of daily treatment (post); †significantly decreased vs. pre-treatment value $P \leq 0.05$; ‡significantly increased vs. pre-treatment value, $P \leq 0.025$.

TABLE II. Clinical chemical alterations in cats associated with the administration of ribavirin (11 mg/kg) by p.o., i.m., or i.v. routes

Variables	p.o.		i.m.		i.v.	
	Pre	Post	Pre	Post	Pre	Post
Alanine aminotransferase (IU/L)	76 ± 10*	104.3 ± 36.4	81.3 ± 9.2	98.3 ± 14.7	55.7 ± 7.8	59.3 ± 8.7
Alkaline phosphatase (IU/L)	64.7 ± 2.8	70 ± 13.1	70 ± 7.4	84.3 ± 12.2	80.3 ± 12.2	71.3 ± 14.5
Creatine kinase (IU/L)	405 ± 88.8	410.3 ± 122.5	544 ± 119.5	313.7 ± 6.1	466.3 ± 104	931.3 ± 787.8
Creatinine (mg/dl)	1.1 ± 0.1	1.2 ± 0.2	1.1 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1 ± 0.1
Glucose (mg/dl)	76.3 ± 3.2	77 ± 7.2	74 ± 5.5	90.7 ± 8.9	72 ± 5	97.3 ± 4.7‡
Urea nitrogen (mg/dl)	26 ± 1	27.7 ± 3.4	28.3 ± 1.8	28.7 ± 2.6	29.3 ± 0.9	21.7 ± 1.8†
Calcium (mg/dl)	9.1 ± 0.3	8.4 ± 0.1†	9.2 ± 0.1	8.4 ± 0.1†	9.3 ± 0.2	8.6 ± 0.2†
Phosphorus (mg/dl)	6.0 ± 0.2	5.2 ± 0.1†	5.8 ± 0.2	4.6 ± 0.2†	5.9 ± 0.2	5.1 ± 0.4
Total protein (g/dl)	6.6 ± 0.2	5.8 ± 0.1†	6.3 ± 0.2	6.1 ± 0.1	6.4 ± 0.4	6.1 ± 0.1
Albumin (g/dl)	3.7 ± 0.2	3.3 ± 0.1	3.8 ± 0.1	3.5 ± 0.1†	3.7 ± 0.2	3.4 ± 0.1
Globulin (g/dl)	2.8 ± 0.2	2.5 ± 0.1	2.4 ± 0.1	2.7 ± 0.1‡	2.7 ± 0.3	2.7 ± 0.2
Sodium (meq/L)	152.3 ± 1.2	148 ± 1†	151.3 ± 1.2	147.7 ± 0.7†	151.7 ± 1.3	148.7 ± 0.3†
Potassium (meq/L)	4.4 ± 0.3	4.6 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.4 ± 0.1	4.3 ± 0.1
Chloride (meq/L)	119.3 ± 0.9	120.3 ± 0.3	115.7 ± 0.9	117.3 ± 1.5	118 ± 0.6	117.7 ± 1.5
Total carbon dioxide (meq/L)	20.4 ± 0.8	20.3 ± 0.7	20.8 ± 1.5	21 ± 1.6	19.1 ± 1.5	18.4 ± 1.3
Anion gap (meq/L)	13.7 ± 1.9	7.6 ± 1.4†	13.5 ± 0.9	9.4 ± 0.7†	15.6 ± 1.9	11.6 ± 0.6†

*Values represent mean ± SE (n = 3 cats for each route) of all observations. Cats were sampled 24 h before administration of ribavirin (pre) and again after 10 days of daily treatment (post); †significantly decreased vs pre-treatment value, $P \leq 0.05$; ‡significantly increased vs. pre-treatment value, $P \leq 0.05$.

TABLE III. Haematologic alterations in cats associated with the administration of ribavirin (22 mg/kg) by p.o., i.m., or i.v. routes

Variables	p.o.		i.m.		i.v.	
	Pre	Post	Pre	Post	Pre	Post
Haemoglobin (g/dl)	12.3 ± 0.7*	11.3 ± 0.7	12 ± 0.5	12.3 ± 0.2	12.7 ± 0.8	14.4 ± 0.1
PCV (%)	38.3 ± 2.0	35.7 ± 2.2	37.3 ± 1.8	39.3 ± 0.9	38.7 ± 2.4	46.5 ± 2.5‡
Erythrocytes ($\times 10^6$/μl)	8.8 ± 0.5	8.0 ± 0.2	8.8 ± 0.6	9.0 ± 0.4	9.2 ± 0.8	9.9 ± 0.1
Leukocytes ($\times 10^3$/μl)	11.3 ± 2.2	9.9 ± 2.3	6.8 ± 0.5	9.2 ± 1.9	8.8 ± 1.0	6.6 ± 1.8
Neutrophils ($\times 10^3$/μl)	6.7 ± 1.2	5.6 ± 1.5	3.4 ± 0.4	5.1 ± 1.7	5.0 ± 1.1	4.7 ± 2.1
Lymphocytes ($\times 10^3$/μl)	3.9 ± 0.6	3.7 ± 0.9	2.9 ± 0.2	3.6 ± 0.6	3.5 ± 0.5	1.5 ± 0.8†
Platelets ($\times 10^3$/μl)	171 ± 19.1	98.7 ± 15.9†	195.7 ± 40.4	115.7 ± 13.9	157.7 ± 20.2	66.7 ± 6.8†
Mean platelet volume (fl)	11.9 ± 0.1	12.2 ± 0.1‡	11.5 ± 0.2	11.9 ± 0.2	11.4 ± 0.3	12.5 ± 0.1‡

*Values represent mean ± SE (n = 3 cats for each route) of all observations. Cats were sampled 24 h before administration of ribavirin (pre) and again after 10 days of daily treatment (post); †significantly decreased vs. pre-treatment value, $P \leq 0.05$; ‡significantly increased vs. pre-treatment value, $P \leq 0.05$.

TABLE IV. Clinical chemical alterations in cats associated with the administration of ribavirin (22 mg/kg) by p.o., i.m., or i.v. routes

	p.o.		i.m.		i.v.	
Variables	Pre	Post	Pre	Post	Pre	Post
Alanine aminotransferase (IU/L)	69 ± 7.2*	87.3 ± 5.0§	75.3 ± 11.3	113 ± 34.2	50 ± 3.2	46†
Alkaline phosphatase (IU/L)	54 ± 11.4	53.7 ± 14.2	63.7 ± 13.0	50 ± 9.9	68.3 ± 14.6	116.3 ± 10.9§
Creatine kinase (IU/L)	407.3 ± 16.3	259.3 ± 18.2‖	299.3 ± 131.3	1191.7 ± 478.4	304.3 ± 107.4	1107.7 ± 515.6
Creatinine (mg/dl)	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.9	1.0 ± 0.1	1 ± 0.1
Glucose (mg/dl)	91.3 ± 4.7	90.7 ± 3.8	87 ± 5.0	100.7 ± 6.9	101 ± 11.0	162†
Urea nitrogen (mg/dl)	27.3 ± 2.4	30.3 ± 3.2	27.3 ± 4.2	24.7 ± 2.3	22 ± 4.0	25.3 ± 2.7
Calcium (mg/dl)	9.9 ± 0.1	9.0 ± 0.1‖	9.6 ± 0.2	9.2 ± 0.1	10.0 ± 0.2	9.9 ± 0.4‡
Phosphorus (mg/dl)	6.2 ± 0.1	6.1 ± 0.2	5.7 ± 0.1	5.7 ± 0.3	6.6 ± 0.3	6.0†
Total protein (g/dl)	6.3 ± 0.1	6.0 ± 0.1	6.4 ± 0.3	6.3 ± 0.1	6.3 ± 0.2	6.4 ± 0.3
Albumin (g/dl)	3.7 ± 0.1	3.5 ± 0.1	3.7 ± 0.1	3.6 ± 0.1	3.8 ± 0.1	3.7 ± 0.2
Globulin (g/dl)	2.6 ± 0.2	2.5 ± 0.1	2.7 ± 0.2	2.7 ± 0.1	2.6 ± 0.1	2.7 ± 0.1
Sodium (meq/L)	148 ± 0.6	150 ± 0.6§	149 ± 1.0	151.3 ± 0.9	149.3 ± 0.7	150.7 ± 0.7
Potassium (meq/L)	4.0 ± 0.1	4.0 ± 0.2	4.0 ± 0.1	3.8 ± 0.1	4.1 ± 0.2	4.3†
Chloride (meq/L)	121.3 ± 0.9	120 ± 0.6	121 ± 1.7	117.3 ± 1.2	118.7 ± 1.3	113.7 ± 3.2
Total carbon dioxide (meq/L)	17.9 ± 0.5	18.5 ± 0.9	19.7 ± 0.5	21.0 ± 0.5	20.5 ± 0.3	21.5†
Anion gap (meq/L)	8.7 ± 0.4	11.4 ± 0.1§	8.3 ± 0.8	12.9 ± 1.0§	10.2 ± 0.9	14.2†

*Values represent mean ± SE (n = 3 cats for each route) of all observations. Cats were sampled 24 h before administration of ribavirin (pre) and again after 10 days of daily treatment (post); †n = 1; ‡n = 2; §significantly increased vs. pre-treatment value, $P \leq 0.05$; ‖significantly decreased vs. pre-treatment value, $P < 0.002$.

TABLE V. Haematologic alterations in cats associated with the administration of ribavirin (44 mg/kg) by p.o., i.m., or i.v. routes

	p.o.		i.m.		i.v.	
Variables	Pre	Post	Pre	Post	Pre	Post
Haemoglobin (g/dl)	12.7 ± 0.2*	13.9 ± 1.6	11.9 ± 0.8	11.6 ± 1.5	12.2 ± 0.6	15 ± 0.5‡
PCV (%)	39.7 ± 0.9	42 ± 5.5	36.3 ± 6.5	35.7 ± 4.7	37.7 ± 1.9	46.3 ± 2.0‡
Erythrocytes ($\times 10^6/\mu l$)	9.3 ± 0.3	9.4 ± 0.6	8.7 ± 0.5	8.2 ± 0.9	8.8 ± 0.5	10‡
Leukocytes ($\times 10^3/\mu l$)	9.3 ± 1.5	6.7 ± 1.2	14.8 ± 5.9	6.5 ± 1.2	10.7 ± 0.7	5.2 ± 1.5†
Neutrophils ($\times 10^3/\mu l$)	5.3 ± 1.0	3.8 ± 0.8	11.9 ± 5.7	4.2 ± 1.1	7.4 ± 0.8	3.0 ± 1.4†
Lymphocytes ($\times 10^3/\mu l$)	3.4 ± 0.9	2.9 ± 0.5	1.7 ± 0.7	2.1 ± 0.7	2.5 ± 0.2	2.0 ± 0.1†
Platelets ($\times 10^3/\mu l$)	202.7 ± 12.7	44.7 ± 5.8†	223 ± 20.0	75 ± 12.6†	147.7 ± 4.3	92.7 ± 23.9†
Mean platelet volume (fl)	12.2 ± 0.5	12.6 ± 0.2	11.7 ± 0.1	12.3 ± 0.2‡	11.4 ± 0.4	12.0 ± 0.2

*Values represent mean ± SE (n = 3 cats for each route) of all observations. Cats were sampled 24 h before administration of ribavirin (pre) and again after 10 days of daily treatment (post); †significantly decreased vs. pre-treatment value $P \leq 0.05$; ‡significantly increased vs. pre-treatment value, $P \leq 0.05$.

TABLE VI. Clinical chemical alterations in cats associated with the administration of ribavirin (44 mg/kg) by p.o., i.m., or i.v. routes

Variables	p.o.		i.m.		i.v.	
	Pre	Post	Pre	Post	Pre	Post
Alanine aminotransferase (IU/L)	76 ± 2*	454 ± 285.6	50 ± 13.4	145.3 ± 36.4‡	50 ± 6.5	211.7 ± 38.7‡
Alkaline phosphatase (IU/L)	69.3 ± 17.6	57 ± 15.7	80.7 ± 3.9	78 ± 2.6	81 ± 2.6	83.3 ± 4.5
Creatine kinase (IU/L)	495.7 ± 140.9	1375.3 ± 461.6	350.7 ± 84.9	412.7 ± 145.3	522.7 ± 111.7	2367.3 ± 830.8‡
Creatinine (mg/dl)	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	0.8 ± 0.1†	1.2 ± 0.1	1.1 ± 0.1
Glucose (mg/dl)	82.7 ± 2.9	76 ± 2.9	76 ± 16.2	110.7 ± 31.3	81.7 ± 4.4	129.3 ± 33.5
Urea nitrogen (mg/dl)	26.3 ± 0.9	20 ± 0.6†	26 ± 3.5	19.3 ± 0.7	28.3 ± 3.0	20.3 ± 0.7†
Calcium (mg/dl)	8.9 ± 0.4	7.9 ± 0.1†	8.4 ± 0.5	8.1 ± 0.2	8.2 ± 0.1	8.6 ± 0.1‡
Phosphorus (mg/dl)	5.5 ± 0.6	4.0 ± 0.2†	6.2 ± 0.4	4.5 ± 0.2†	5.3 ± 0.3	5.1 ± 0.2
Total protein (g/dl)	6.3 ± 0.1	5.4 ± 0.1†	6.0 ± 0.2	5.7 ± 0.3	6.1 ± 0.1	5.9 ± 0.1
Albumin (g/dl)	3.7 ± 0.1	3.2 ± 0.1†	3.4 ± 0.1	3.2 ± 0.1	3.6 ± 0.1	3.4 ± 0.1
Globulin (g/dl)	2.6 ± 0.1	2.2 ± 0.1†	2.6 ± 0.2	2.5 ± 0.2	2.7 ± 0.2	2.5 ± 0.1
Sodium (meq/L)	153 ± 1	152 ± 2.1	152.7 ± 0.3	151 ± 1	152.3 ± 0.7	153.7 ± 0.9
Potassium (meq/L)	3.9 ± 0.2	3.9 ± 0.3	4.2 ± 0.1	3.9 ± 0.2	4.6 ± 0.1	4.2 ± 0.2
Chloride (meq/L)	122.3 ± 0.9	122.3 ± 3.2	120.7 ± 1.3	120.7 ± 2.6	120 ± 1	121 ± 0.6
Total carbon dioxide (meq/L)	20.4 ± 0.7	21.5 ± 2.2	20.1 ± 0.8	20.5 ± 0.8	19.5 ± 1.2	20.3 ± 0.5
Anion gap (meq/L)	10.3 ± 1.2	7.9 ± 1.2	11.8 ± 2.4	9.8 ± 1.1	13.1 ± 1.2	12.6 ± 0.4

*Values represent mean ± SE (n = 3 cats for each route) of all observations. Cats were sampled 24 h before administration of ribavirin (pre) and again after 10 days of daily treatment (post); †significantly decreased vs. pre-treatment value, $P \leq 0.03$; ‡significantly increased vs. pre-treatment value, $P \leq 0.05$.

in contrast, were significantly ($P = 0.03$) increased in the i.v. group. Total protein and albumin levels were reduced in all groups and were significantly ($P < 0.025$) decreased in cats treated orally vs. cats treated i.m. or i.v. The anion gap was reduced and the blood glucose concentration increased in all groups. Increases in BUN or creatinine were not observed.

Bone marrow changes

A dose-dependent effect of ribavirin on bone marrow cytopathology was observed (Table VII). Bone marrow changes generally included erythroid and megakaryocytic (more rarely, myeloid) hypoplasia, myeloid left shift, increased M:E ratio, and abnormalities in megakaryocyte morphology (e.g., asynchronous maturation, atypical nuclear lobulation). Bone marrow smears taken pre- and post-treatment (PTD 10) demonstrated that two of nine cats (1 p.o., 1 i.m.) given 11 mg of ribavirin/kg developed mild hypoplasia of megakaryocytes, and another cat had an increased M:E ratio compared to baseline. Two cats given 22 mg of ribavirin/kg i.m. had moderate megakaryocytic hypoplasia, and one cat given this dose of ribavirin i.m. had atypical nuclear lobulation of megakaryocytes. Three cats (2 p.o., 1 i.m.) had a moderate amount of erythroid hypoplasia. All cats that received 44 mg of ribavirin/kg had pronounced megakaryocytic and erythroid hypoplasia and all had an increased M:E ratio. Six of nine cats given ribavirin at this dose had atypical nuclear lobulation of megakaryocytes and seven cats had asynchronous megakaryocyte maturation.

Pathologic changes

All cats were well-nourished and they had moderate-to-abundant stores of adipose tissue. Livers from 12 of 27 cats had mildly reticulated patterns, usually associated with a paucity of ingesta in the gastrointestinal tract. Affected cats were evenly distributed among treatment groups. Three of nine cats given 22 mg of ribavirin/kg (1 p.o., 2 i.m.) had mildly dilated lateral ventricles of the brain. Seven of 27 cats had mild enteritis or colitis, including

TABLE VII. Bone marrow cytopathology in cats given various doses of ribavirin

	No. of cats with:				
Group*	MK hypoplasia	MK dysplasia	Asynchronous MK maturation	Erythroid hypoplasia	Increased M:E ratio
11 mg/kg					
p.o.	1	0	0	0	1
i.m.	1	0	0	0	0
i.v.	0	0	0	0	0
22 mg/kg					
p.o.	0	0	0	2	3
i.m.	2	0	0	1	0
i.v.†	0	1	0	0	1
44 mg/kg					
p.o.	3	3	3	3	3
i.m.	3	1	2	3	3
i.v.	3	2	2	3	3

*$n = 3$ cats at each route for each dose; †only one cat evaluated (postmortem samples of two cats were unsuitable for cytology). Abbreviations: MK = megakaryocyte; M:E = myeloid: erythroid ratio.

four cats dosed p.o. (one at 11 mg/kg, two at 22 mg/kg and one at 44 mg/kg), two cats treated i.m. (both at 22 mg/kg) and one cat treated i.v. (11 mg/kg). Histologically, specific toxic changes were restricted to the liver, intestinal tract and brain. Hepatic hydropic or fatty degeneration, vacuolation, and centrilobular necrosis occurred in seven cats dosed at 22 or 44 mg/kg. Seven cats had enterocolitis. The intestinal inflammatory lesions consisted predominantly of mononuclear infiltrates in the lamina propria and mucosal microabscesses. These lesions were observed at all dosages but occurred most often in the p.o. groups. Peyer's patches frequently were large and abundant with very active germinal centers. Three cats had dilatation of the cerebral lateral ventricles without hydrocephalus. Two cats (A6 and A4) treated i.v. with 22 mg of ribavirin/kg and given daily injections of ketamine died still under anaesthesia at PID 6 or 9, respectively. Neither cat showed gross or microscopic changes suggestive of toxicosis. Cat A6 had multifocal haemorrhages in the thymus, moderate lymphoid hyperplasia and mild hepatic congestion and hydropic change; cat A4 had mild to moderate lymphoid hyperplasia.

DISCUSSION

Ribavirin toxicity to rodents, non-human primates (rhesus monkey) and humans has been studied previously (Hillyard, 1980; Canonico, 1983; Canonico *et al.*, 1984a). Administration of ribavirin for 10 days at daily dosages up to 30 mg/kg produced mild normocytic anaemia in rhesus monkeys, while higher dosages ($\geq$ 50 mg/kg/day) resulted in severe anaemia in the absence of a reticulocyte response. The data indicated that the anaemia seen with lower dosages was likely caused by accelerated red cell destruction, probably within the mononuclear phagocyte system. An additional cause of anaemia observed at higher dosage and confirmed by bone marrow biopsy was inhibition of erythropoiesis (Huggins, 1989). In humans, relatively low dosages of ribavirin (1 g per day) caused unconjugated bilirubin levels to double and the reticulocyte count to increase (Smith & Charette, 1980), while much higher dosages (3.9–12.6 g per day) caused decreases in haemoglobin levels (Fernandez, 1980). These changes were reversed rapidly after treatment was discontinued.

There is considerable species variation in sensitivity to the haematologic toxicity of ribavirin. It is known that red blood cells sequester large amounts of ribavirin (Canonico *et al.*, 1984b); the half-life of ribavirin in red cells is about 40 days (Nicolson, 1984). The sensitivity of various species to the haematologic effects of the drug is related to the amount of ribavirin sequestered. For example, mice and rats, which accumulate markedly less ribavirin in red cells than do humans or monkeys, are much more resistant to the drug's haematologic toxicity (Huggins, 1989); mice have been given 45–340 mg of ribavirin/kg daily without noticeable toxicity (Durr *et al.*, 1975). In monkeys and rats, ribavirin is mutagenic, teratogenic, embryotoxic, and possibly, carcinogenic (Reines & Gross, 1988). The lethal dose of ribavirin in cats and its specific toxic effects presently are not known.

In the present study of cats, the most important toxic effects of ribavirin were progressive anorexia and weight loss, mild pallor of mucous membranes, severe thrombocytopenia, and variable decreases in WBC and neutrophil numbers. Some cats, particularly those receiving 11 or 22 mg of ribavirin/kg/day, also had decreases in red cells, haemoglobin and haematocrit. Other cats, notably those given the higher dosages of ribavirin, had significant increases in liver-specific enzymes. Other changes in clinical chemistry profiles post-treatment were variable and generally were not exceptional. There was a tendency, however, for blood calcium levels and plasma proteins to decline (particularly in the p.o. groups), and there were mild fluctuations in electrolytes, notably sodium and glucose. Seemingly, decreases in plasma proteins and electrolytes may have been related to persistent anorexia or possibly to binding of these molecules to the drug in the gastrointestinal tract or blood. The increase in glucose observed in all groups of cats given the highest dosage of ribavirin was unexpected; possibly, this increase was induced by severe physiological stress and endogenous adrenocorticosteroid release, resulting in increased

glycogenolysis and gluconeogenesis. The reason for the variable fluctuations in the anion gap and the overall influence, if any, of ionic charge contributed by the ribavirin on acid-base balance were not clear. Although the influence of giving multiple doses of ketamine in the i.v. group on clinical chemistries was not specifically evaluated, there were no significant differences between groups in clinical chemistry profiles other than an increase in serum calcium in cats given ribavirin i.v. at 44 mg/kg vs. those given ribavirin p.o. or i.m. that would suggest a significant effect.

Histologically, cats showed hepatocellular degeneration, suggesting some mild hepatotoxicity. Dilatation of the cerebral lateral ventricles was observed in three cats given the medium dose (22 mg/kg) of ribavirin p.o. or i.m. It was not apparent whether this was an incidental finding or was associated instead with ribavirin toxicity; there were no other neuropathologic changes or abnormalities in blood glucose or other electrolyte or blood protein concentrations that might help explain this lesion. Curiously, the brain lesion was not observed in cats given higher dosages of the drug. Bone marrow biopsies frequently revealed marked decreases in megakaryocytes, disturbances in megakaryocyte maturation and morphology, and erythroid hypoplasia. In contrast to our findings in cats, monkeys given ribavirin in a previous study had a dose-related increase in platelets, megakaryocytic hyperplasia, and no significant effects on WBC counts (Canonico *et al.*, 1984a,b; Cosgriff *et al.*, 1984). Many of the ribavirin-treated cats in our study that had lesions of the bone marrow, including megakaryocytic and/or erythroid hypoplasia, did not demonstrate significant reductions in red cells or show clinical evidence of haemorrhage or anaemia. The exact reason for this was unclear; however, the absence of anaemia may have been related to the half-life of circulating erythrocytes in cats (76 ± 0.9 days; Weiser, 1989) and the length of the study, whereas the absence of haemorrhagic diathesis in the face of severe thrombocytopenia may have been related to compensatory platelet mechanisms, e.g. increases in mean platelet size, a morphologic alteration observed in many of the ribavirin-treated cats in this study. Similar compensatory mechanisms in thrombocytopenic cats with viral-induced disseminated intravascular coagulopathy have been described previously (Weiss *et al.*, 1980).

Considering the marked thrombocytopenia after 10 days of treatment and the bone marrow lesions (viz. hypoplasia of erythroid and platelet precursors), a haemorrhagic diathesis or anaemia (or both) might have been expected to occur in the ribavirin-treated cats if the treatments were continued. Unfortunately, because the cats of this study were euthanatized after 10 days of treatment, the duration of the drug-induced bone marrow and haematologic changes and their reversibility after cessation of therapy were not determined. Previous studies of ribavirin in humans and monkeys indicated that most if not all of the toxic effects of the drug on blood cells and bone marrow were transient and reversed rapidly after treatments were discontinued (Fernandez, 1980; Canonico *et al.*, 1984a,b; Cosgriff *et al.*, 1984).

In a previous study of ribavirin in specific-pathogen-free cats infected experimentally with FCV, Povey (1978b) described toxic changes attributed to ribavirin which were similar to those described in this report, viz. pronounced weight loss, severe thrombocytopenia, and decreases in total WBC and neutrophils. Some of the cats in the former study had severe internal haemorrhages and depression of red blood cells, PCV and haemoglobin, while others had mean increases in ALT concentrations. Although some of the lesions may have been induced by virus, because cats treated with ribavirin were also infected concurrently, the author in fact concluded that the toxicity of ribavirin had exacerbated FCV clinical disease. Because the dosage of ribavirin (75 mg/kg) used to treat the FCV-exposed cats of the previous study was between 2–7 times higher than dosages used in this study and because the haematologic effects described in the previous study were largely reversed within 1 week of cessation of therapy, we believed that the drug-induced haematologic toxicity in the cats of this study probably would have subsided had the treatments been discontinued.

Our results indicated that ribavirin may cause acute toxic effects, even at low dosages, on feline bone marrow megakaryocytes and erythroid precursors, resulting in pro-

nounced thrombocytopenia and potentially anaemia. Conceivably, suppression of bone marrow platelet and erythroid precursors may predispose ribavirin-treated cats to anaemia and/or bleeding tendencies. The clinical benefits, if any, of using ribavirin as antiviral therapy for cats suffering from viral diseases (e.g. FeLV infection, feline infectious peritonitis), which may themselves induce bone marrow hypoplasia, thrombocytopenia, or haemorrhagic diathesis, may be outweighed by the drug's toxicity. Indeed, ribavirin toxicity can exacerbate lesions and disease induced by viruses, as reported in mice infected with cytomegalovirus (Dowling *et al.*, 1976) or cats infected with FCV (Povey, 1978b) or feline infectious peritonitis virus (Weiss *et al.*, in press).

The pathogenesis of ribavirin toxicity in the cats of this study was not fully understood. Ribavirin apparently can suppress cellular DNA synthesis and also inhibit translation of proteins via inhibition of 5′ cap formation of mRNA (Reines and Gross, 1988), and it is cytotoxic to resting cell lines at concentrations *in vitro* of 200–1000 μg/ml (Nicholson, 1984). The predominant toxicity in cats that we observed was suppression of precursor cells in bone marrow, particularly megakaryocytes and myeloid and erythroid cells. It seems likely that this effect is dose-related and thus may be controlled somewhat by lowering the dose of ribavirin administered. However, lowering the dose of drug unfortunately may compromise its antiviral efficacy. Another approach to reduce drug toxicity without jeopardizing antiviral efficacy is to incorporate smaller doses of drug within liposomes that target the drug more specifically to sites of virus replication, thereby substantially lowering the therapeutic dose (Alving, 1983). Alternatively, concurrent administration of particular recombinant growth factors, e.g. granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL)-3, or erythropoietin may be considered along with the ribavirin therapy to enhance bone marrow function and protect cats against the specific toxic effects of the ribavirin. For example, IL-3 has been used to decrease chemotherapy-induced myelosuppression, and sequential administration of IL-3 and GM-CSF has been shown to cause a marked increase in white blood cells, including myeloid lineages and platelets (Ogilvie, 1991).

Results indicated that ribavirin administered daily to healthy cats at a dosage of 11 to 44 mg/kg p.o., i.m., or i.v. for a week or more was associated with variable amounts of bone marrow and haematological toxicity, anorexia and weight loss. Additional toxicologic studies of ribavirin using even lower dosages than we reported (i.e. < 11 mg of ribavirin/kg) should be performed before evaluating this antiviral drug clinically in cats.

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